The Influence of Caries Infiltrant Combined with and without Conventional Adhesives on Sealing of Sound Enamel (*In Vitro* Study)

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ABSTRACT

Background: The formation of white spot lesions around fixed orthodontic attachments is a common complication during and after fixed orthodontic treatment, which hinders the result of a successfully completed orthodontic treatment. The aim of the study was to assess the effectiveness of the Caries Infiltrant (ICON®) on prevention of caries on the smooth enamel surface when applied alone or combined with conventional adhesives.

Materials and methods: Seventy eight human premolar enamel discs were randomly assigned to six groups (n=13). The discs were etched and treated with resins of different monomer content forming the following groups: (1)Untreated etched samples served as the negative control, (2) ICON® (DMG), (3) Adper™ SB 2 (3M ESPE), (4) Heliobond (IvoclarVivadent), (5) ICON®+ Adper™ SB 2 and (6) ICON®+ Heliobond. Specimens were subjected to demineralization by immersion in hydrochloric acid (pH 2.6) for 18 days. Calcium dissolution into the acid was assessed by photometric test via spectrophotometer at 24 hour intervals.

Results: The results revealed that, there was a highly significant difference between the sealed groups and the unsealed (untreated) one ($p \leq 0.00$) indicating that the unsealed specimens showed the highest amount of Ca ion loss among all other groups. Additionally, there was no significant difference between untreated specimens and the ICON® sealed ones. While, Heliobond decreased the Ca ion loss significantly compared to the untreated specimens and Adper™ SB 2 performed significantly better than both ICON® and Heliobond. Furthermore, the combination of ICON® with either Adper™ SB 2 or Heliobond served as the best protective measures and maintained the protective effect during the whole experiment period. Therefore, within the limitations of this in vitro study, it could be concluded that the use of Caries Infiltrant prior to the application of the tested conventional adhesives increases their protective effect against demineralization.

Key words: White spot lesions, sealants, Infiltrants, ICON®. (J Bagh Coll Dentistry 2016; 28(2):119-125).

INTRODUCTION

The fixation of orthodontic brackets enhances plaque retention and this favors the development of demineralization and initial caries around the brackets when the oral hygiene of the patient is poor [(1)].

Studies have shown that, compared with non-orthodontic patients, orthodontic patients are much more vulnerable to the demineralization of enamel with a rate of 4.9% to 84% [(2)].

As carbohydrates consumed daily, they are fermented by the bacteria that colonized in the plaque and lead to decrease the intraoral PH. The low PH results in dissociation of calcium and phosphate ions from enamel in an attempt to reach chemical equilibrium in the oral environment [(3,4)]. It was found [(5,6)] that 24% and 11%patients, respectively, with existing WSLs at the time of bracket fixation. In addition to that,[(7)]the prevalence of these acquired surface lesions due to orthodontic treatment is relatively high, affecting more than 40% to 60% of patients. Also, these lesions can appear very rapidly, as fast as in a couple of weeks after the placement of brackets [(8)]. Therefore, different attempts have been made to increase the caries resistance of enamel prior to and during fixed orthodontic treatment (e.g. Application of fluoride or casein-phosphate-amorphous).

Other preventive measures are chlorhexidine mouth rinses, ozone applications, probiotics, xylitol, and sealants [(9,10)].

Another suggestion was about the use of an adhesive patch as an intermediate layer under metal brackets to prevent demineralization under and around the bracket to relocate the bracket-patch margins to areas that are easier to access for oral hygiene [(9)]. Additionally, many studies [(10,11)] discussed the use of different (fluoridated) bonding agents and sealants as a means to protect the surrounding enamel from demineralization.

Previously, many authors [(4,12)] stated: "Preventive measures that do not require patient compliance are considered more predictable since only 13% of the patients were reported to achieve excellent cooperation with the use of mouth rinses and tooth brushing". While [(13,14)] remineralization of (WSLs) do not disappear unless they are removed mechanically by abrasion, etching, or masking by resin infiltration or treated in a restorative fashion.

Therefore, [(9,15)] sealing the susceptible enamel prior to bracket bonding in order to form a caries-protective shield has been the focus of interest in

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previous studies that primarily intend to eliminate patient compliance.

In principle, sealants cover the whole buccal surface adjacent to brackets, forming a physical barrier. This protective shield is subjected to physical challenges such as acid attacks from bacterial plaque and acidic soft drinks as well as daily tooth brushing, which might impair the seal [4,8,9,15].

In recent years, many Clinical trials [16,17] showed that, the caries progression of lesions infiltrated with a low-viscosity resins that have a high capability for penetrating initial carious lesion is significantly reduced. But, it was [18,19] found that, although resin Infiltrants were originally developed for penetration into carious lesions and occlusion of diffusion pathways, they also prevented enamel surface demineralization to some extent. In contrast, to conventional sealants, where the physical barrier remains on the enamel surface as a covering coat, this Infiltrant presents rapid capillary penetration into the pores creating a diffusion barrier within the enamel with very low-viscosity and superior surface wetting abilities. In addition to that, new retention areas for plaque accumulation at the sealed margins are being avoided**.**

However, in spite of the deeper penetration of carious lesions where is a porous structure for the resin to infiltrate, it has not been shown if this resin can infiltrate phosphoric acid etched sound enamel where only limited capillary diffusion is imaginable [20]**.**

Very little information have been gained regarding the effect of caries Infiltrants on human teeth when they subjected to orthodontic treatment. Therefore, it is intended to implement this study in this field of dentistry.

MATERIALS AND METHODS

Sound human premolars, two conventional adhesive resins (Adper ™ Single Bond 2 and Heliobond) and low- viscosity caries Infiltrant ICON® were employed in this in vitro study. The adhesives used were conventional bonding agents with different ratios of Bis-phenol-A-glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) content. The low-viscosity caries infiltrant was TEGDMA-based. The chemical compositions of the materials are summarized in **Table 1**.

Study design:

Seventy eight enamel discs cut from the buccal aspect of each collected premolar just below the buccal cusp tip to get a valid thickness of enamel. The discs randomly assigned into 6 groups (n=13) to be sealed with different bonding agents. These agents were conventional bonding agents and Infiltrant with different ratios of (Bis-GMA) and (TEGDMA). Unsealed control, caries infiltrant, conventional orthodontic bonding agent (Adper ™ Single Bond 2), unfilled bonding agent (Heliobond), caries infiltrant+ conventional orthodontic bonding agent, and caries infiltrant+ unfilled bonding agent.All the discs were subjected to 18 days acidic challenge for demineralization and Ca ion conc. was measured every 24 hrs using photometric test for Ca ion via spectrophotometer.

Sample preparation:

Collected teeth were cleaned from blood and gross debris by rinsing with tap water [21]. Then, they were polished with oil free pumice and prophylactic rubber cup (five teeth per one cup) adjusted to a low- speed hand-piece. They were dried and tested by magnifying lens and light cure illumination for detection of cracks and pre-existing defects [22,23].Then, stored in 0.9 % NaCl, for no longer than two months to prevent dehydration and cross contamination**.** The stored and cleaned premolars milled and shaped to have a (3mm diameter and 2mm thickness) by using torna and micro-engine under water cooling.The buccal surface ground flat with full diamond disc. All the cutting procedure involved water cooling. Then discs embedded in a costume made silicone cylindrical mold (6mm in diameter and 6mm in depth). The embedded polished with rubber cups and oil free pumice, cleaned with air/ water syringe and stored in double recycled distilled water thereafter.

Sealing procedure:

After dying (15 sec. for each specimen), all specimens were etched with 38% phosphoric acid gelfor 20 sec. and then rinsed with copious amount of water for 30 sec. the bonding procedure for all of the specimens (except the unsealed group) as in the following manner:

1- Caries Infiltrant: ethanol (Icon-Dry) was applied by using the manufacturer syringe for 30 sec. and dried for 10 sec. Then, the low viscosity caries Infiltrant (ICON® Infiltrant) was emptied in dippen dish. It was applied in one coat with a micro-brush and let set for 3min., then light cured for 60 sec.; a second layer was applied, let set for 1 min., and light cured for 40 secaccording to manufacturer information.

2- Conventional bonding agent: Adper™ Single Bond 2. Two coats of bonding agent were applied with a fully saturated micro-brush, while massaging in over the entire surface for 15 sec. Air thinning for 5 sec. to evaporate the solvent and light cured for 10 secaccording to manufacturer information.

3- Unfilled bonding agent: Heliobond was applied in one coat with a micro brush and light application of an air jet for 5 sec. Then light cured for 10 secaccording to manufacturer information.

4- Caries Infiltrant + conventional bonding agent: steps 1 and 2 were repeated subsequently.

5- Caries Infiltrant + unfilled bonding agent: steps 1 and 3 were repeated subsequently.

All procedures involving air/water jet and light curing unit were performed using a custom-made devices assuring standard distance to the specimens from the application tips of triple syringe and light curing unit. The distance was 2 cm from air/water syringe tipstoeach specimen. The intensity of the light was 1600 mW/cm² at light guide tip; 1110 mW/cm² at 4 mm distance from the specimen. The irradiance of light was checked by a radiometer (DigiRate – Radiometer, LM-100, Monitex, Taiwan). The light-curing unit was checked at the beginning and after five applications for constant output. Following these pretreatments, the specimens were stored in distilled water for 24 hours at 37 °C for complete polymerization.

Acidic challenge and evaluation of sealing ability:

All specimens were immersed in hydrochloric acid (pH 2.6) for 18 days. Sealing ability was quantified by the amount of Ca released from the specimens into the acid solution using photometric test for Ca via spectrophotometer. Twenty µL of the acid solution was added to the test tubes and mixed with 1 mL of the color reagent (CA ION liquicolor 200ml complete test kits, Human Gmbh Max-Planck-Ring 21, 65205 Wiesbaden – Germany). Absorbance was read at 570 nm in the Spectrophotometer reader. The measurements were performed at a room temperature of 25° C according to manufacturer information of Ca ion kit.

Table 1: Composition of the low-Viscosity caries Infiltrant and the conventional bonding resins according to the manufacturers' information.

Product	Chemical compositions	Manufacturer
1) ICON® (Infiltrant) 0.45 ml 2) ICON® (Dry)	TEGDMA-based resin matrix, Initiators – additives. Ethanol.	DMG, Hamburg, Germany; batch 220402
Adper™ single bond2 6g	BisGMA 10%-20% ,HEMA 5% , Nanofillers 10%-20%, dimeth-acrylates, water, a novel photo-initiator system and a methacrylate functional copolymer of polyacrylic and Polyitaconic acids, Ethanol 25%-35%.	3M ESPE, St. Paul, MN U.S.A; batch N435767
Heliobond 6g	Bis-GMA 50% - 100% TEGDMA 25%- 50% Initiators – stabilizers.	IvoclarVivadent, Schaan, Liechtenstein; batch P82828

RESULTS

Data were collected and analyzed using SPSS (statistical package of social science) software version 15 for windows XP Chicago, USA.The unsealed samples showed higher mean values of Ca ion conc. than all other tested samples during every day of the estimated period (18 days). ANOVA showed a significant difference ($P < 0.000$) among the mean values of Ca ion conc. of the test's groups in each tested day and during the whole tests period.LSD showed that there was a highly significant difference between the sealed groups and the unsealed one ($p \leq 0.00$) except between (G1: unsealed group and G2: ICON® Infiltrant sealed group) there was no significant difference. While, there was a significant difference between (G2: ICON® Infiltrant sealed group) and the other sealed groups except for the (G4: Heliobond sealed group), with no significant difference was present between them. Furthermore, there was no significant difference between (G3: Adper™ Single Bond 2 sealed group) and the other sealed group. But, a significant difference found between the (G4: Heliobond sealed group) and the combined group (G5: Adper™ Single Bond 2 + ICON® sealed group), while there was no significant difference between G4 and (G6: Heliobond + ICON® sealed group). Also, between the two combined groups there was no significant difference (**Table 2**), (**Figure 1**).

Table 2: Descriptive statistics and groups comparison of the daily Ca ion conc. during the whole study period (18 days).

Groups	Mean	S.D.	Min.	Max.	F-test	p-value
G1	2.08	0.65	0.95	2.92	7.745	0.000 (HS)
G2	1.73	0.66	0.71	2.67		
G3	1.16	0.72	0.28	2.34		
G4	1.39	0.74	0.33	2.53		
G5	0.93	0.64	0.08	1.96		
G6	1.03	0.67	0.17	2.19		

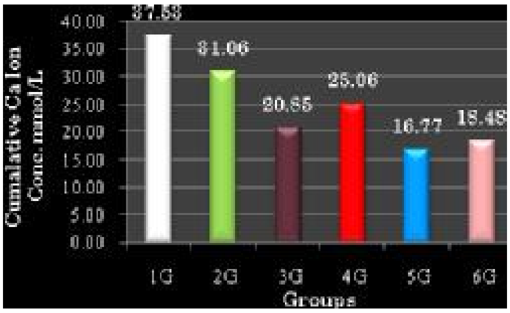

Figure 1: Cumulative Ca ion conc. in mmol/L over 18 days of acidic challenge revealed that unsealed specimens (G1) released the highest amount of Ca ion while, the other specimens released the less specially the combined ones (G5 and G6).

DISCUSSION

Enamel decalcifications affect many orthodontic patients. These decalcifications named as WSLs; they are caused by inadequate oral hygiene that leads to plaque accumulation around orthodontic appliances. Other factors that predispose a patient to WSLs are appliance design, cement lute failure, poor salivary flow and composition, enamel susceptibility, and dietary practices [24].

In the past, many attempts aimed to minimize or prevent the appearance of these WSLs throughout or after finishing orthodontic treatment, one of those attempts was the invention of low-viscosity resins that called Infiltrants.

Regarding the current study, this Infiltrant represented by ICON® which is an innovative product for the micro-invasive treatment of early cariogenic lesions in the proximaland vestibular regions. It can be used to treat caries in a timely manner without drilling. The proximal version of the product is specifically developed for preserving treatment of incipient proximal caries while, the vestibular version is particularly suited for orthodontic patients after braces removal.Many in vitro studies chose bovine teeth to test the ICON® material but, very little studies dealt with extracted human teeth. Human and bovine enamel have similar radio densities.However, their enamel structure may still differ significantly [25]. Therefore, in the current in vitro study, freshly extracted human premolars had been selected for evaluation of the adhesive systems and their enamel sealing ability against demineralization to provide a comparable environment to human oral cavity during orthodontic treatment and to increase the reliability of the study results. Additionally, a lot of teeth were selected to overcome the limited ability of getting more than one specimen with enough sound enamel (thickness and diameter) from each selected tooth.

The current study demonstrated that, the application of the conventional adhesives alone or combined with the Infiltrant material were more effective than the application of caries Infiltrant alone in protection sound enamel from further demineralization. While, there was no significant difference between the infiltrant sealing alone and no sealing at all.

The Adper™ Single Bond 2 bonding agent was superior in reduction of enamel demineralization compared to the aforementioned groups (ICON®+ Unsealed groups) and to the Heliobond bonding agent, when applied alone. In addition to that, its protective potential against enamel demineralization was improved when combined with the caries Infiltrant. Furthermore, this combination was more effective than the combination with the Heliobond bonding agent and both of them when applied alone or combined with the Infiltrant provided better protection against acidic attack than the unsealed specimens and the Infiltrant sealed ones. This might be due to the chemical compositions of the adhesive systems that determine their clinical success [26].

The presence of nanofillers in 10% weight of the relatively low viscosity adhesive (Adper™ Single Bond 2) promoted the development of a uniform adhesive film and "stabilized" the hybrid layer [27]. Their smaller size, improves the adhesive wet-ability [28]. While, Heliobond represented the unfilled enamel bonding agent as it exhibits potential to penetrate at least early enamel lesions [29,30]. And, the high Bis-GMA content gave the viscose nature of the adhesive and decreases the surface wet ability property, which might result in weakened plugging of the porosities [31-33]. However, this property might be participated in lesser sealing ability than the Adper ™ Single Bond 2 bonding agent when applied alone or combined with the Infiltrant material. Regarding the ICON® it was a TEGDMA- based. The high TEGDMA content and ethanol in adhesives were shown to increase the capillary penetration and wetting ability of the resins, facilitating better micromechanical unity with the enamel. And that'sone of the important prerequisite in enamel sealing [21]. Another suggestion that the superficial penetration and surface coating of the adhesive might be more effective in protecting enamel dissolution than the penetration of the Infiltrant. Furthermore, many studies [21, 31, 34,35] showed that there is a side effect of the high TEGDMA content in the resin matrix because it leads to increases polymerization shrinkage and stress, resulting in lower physical properties. Similarly, more oxygen inhibition and polymerization shrinkage of the low viscosity caries Infiltrant were reported to create heterogeneous areas within the penetrated material, resulting in insufficiently filled porosities of the surface.

In that respect, ICON® with the highest TEGDMA content among the tested resins was expected to provide better penetration into the enamel with higher contact area. In addition, voids in sealant surface due to the oxygen inhibition and polymerization shrinkage were anticipated.

Wetting of enamel with ICON® prior to Heliobond or Adper™ Single Bond 2 primers performed better sealing than all of the single applications, may be due to the incapability of capillary penetration of the two more viscous resins was compensated by ICON®, resulting in a highly protective layer against demineralization. It was found [36] that, the infiltration of the demineralized subsurface layer and the sealing of the surface might have an additive effect on the dissolution protection. And the bonding of the adhesive is not impaired on infiltrated enamel surfaces.

Moreover, covering infiltrated lesions with an adhesive layer might be beneficial in terms of surface properties, as surface roughness of infiltrated lesions is comparatively high [32]. Very few studies evaluated the effect of ICON® on enamel sealing following phosphoric acid etching. One of those studies [20] who compared between the sealing ability of deferent adhesives and ICON®, but they selected bovine teeth to test the enamel protection under acidic challenge. Their findings were similar to the current study in that the use of low-viscosity caries Infiltrant prior to application of the tested conventional adhesives increases their protective effect against demineralization. However, a similar reduction effect on apatite dissolution following hydrochloric acid etching on both sound and demineralized enamel postulated recently [19]**.** In particular, Heliobond alone and its combination with ICON® performed superior than the Infiltrant application alone as found in the current study. But, in contrast to the present findings, combining ICON® with Heliobond did not provide better protection than Heliobond alone. The reason for this declared that, it might be attributed to the extensive etching effect of 120 seconds of hydrochloric acid application, as assumed according to manufacturer kit, which was primarily intended to create a permeable outer layer in the presence of WSLs [37]. In the present study, the application of caries infiltrant following 38% phosphoric acid etching on sound enamel prior to orthodontic bonding procedure could be an alternative preventive measure against WSLs formation, since acid etchant demineralizes the hydroxyapatite crystals of enamel rods, and exposes micro pores on the enamel allowing the adhesive material to interlock and seal the mineralized tissue underneath against acidic challenges resulting from dietary intake of carbohydrates and soft drinks [8,38, 39].

According to the present findings, treated specimens with the caries Infiltrant system followed by the adhesive exhibited significantly lower Ca ion release than the adhesive or the Infiltrant alone and which lead to the following conclusion that the combining procedure provided better sealing against acidic attack [19,20]. Penetration time is another important factor to determine the rate of resin impregnation and plugging the gaps formed by etching. ICON® was the only resin with prolonged penetration time, whereas Heliobond and Adper™ Single Bond 2 bonding agents were photo-polymerized right after their application on the etched surface. This factor might play another role in increasing the sealing property. Clinically, a 180-second application time of ICON® might not be easy to obtain, especially on the buccal surfaces of posterior teeth when the patient is in a supine position, because of saliva contamination. Therefore, allowing the resin to penetrate as long as possible prior to photo-polymerization should be acknowledged as an improving factor [40].

In the present study, the acid attack was applied continuously, mimicking an estimated time period of 9 months with 18 days of continuous pH exposure that resemble to at least 20min/day of acidic challenge. The pH of the acid used was significantly lower than that of the organic acids produced by bacteria. This lower pH was used to increase the quantity of Ca ion dissolved to generate detectable amounts in short time periods and to assure the duration of acidic challenge to represent at least the estimated time period [20].

On the other hand, it's important to mention that the daily acidic pH attack (frequency and magnitude) depend on many variables, such as frequency of sugar intake, percentage of sugar in the food, and properties of saliva and intraoral flora, which show a great diversity among individuals. However, it should be noticed that the current in vitro studyenvironmentdiffered from the in vivo situation in that there was no protective salivary pellicle and enamel surfaces were in continuous contact with the acidic challenge [19].

Another important issue declared in another study that, the sealants performing well under these highly demineralizing conditions would also be able to show the same relative protective effects against demineralization caused by weaker acids. One limitation regarding the demineralization cycle might be that, no remineralization by saliva or other regular protective measures such as the use of fluoride-containing toothpaste has been applied [20]. The rationales behind this approach were to increase the precision of the measurement method by eliminating possible Ca ion contamination from the toothpaste and avoid possible interactions between measurements. A secondary objective was to simulate the worst case scenario for demineralization without the presence of preventive measures.

With these aspects in mind, the endurance of the protective effect provided by ICON® + Adper™ Single Bond 2 and ICON® + Heliobond was anticipated to last throughout the whole course of orthodontic treatment since their seal present the least amount of Ca ion release among all groups through the 18 days acidic challenge, representing approximately 9 months in vivo.

It's worth to mention that, the data for enamel demineralization found in the current in vitro study have to be carefully transferred to the clinical situation except for the used acid. The pellicle formation on the enamel surface resulting in a diffusion barrier might have an effect on mineral loss within the softened enamel according to the findings of deferent studies [41,42].

A series of studies have demonstrated in situ the efficacy of the pellicle in reducing demineralization. Thus, it can be speculated that demineralization of softened enamel in vivo is smaller than found in the present in vitro study due to the buffering capacity of saliva that might decrease the demineralization potential of acidic drinks [43, 44]. Besides the variable tested, other variables must be taken into account in future studies for developing strategies to minimize dental demineralization around the orthodontic brackets.

As conclusions

1- The low viscosity caries infiltrant did not protect enamel from demineralization when applied alone.
2- The low viscosity caries Infiltrant (ICON®) and the 2 conventional bonding agents (Adper™ Single Bond 2 and Heliobond) reduced enamel demineralization when applied alone.
3- The conventional bonding agent (Adper ™ Single Bond 2) with its low viscosity and nanofillers contents provided a higher protection compared to the (Heliobond) with its higher viscosity
4- Both conventional bonding agents provided better sealing when combined with the low viscosity Infiltrant.
5- The combination caries infiltrant with Adper™ Single Bond 2 provided better enamel sealing than the combination with Heliobond.

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